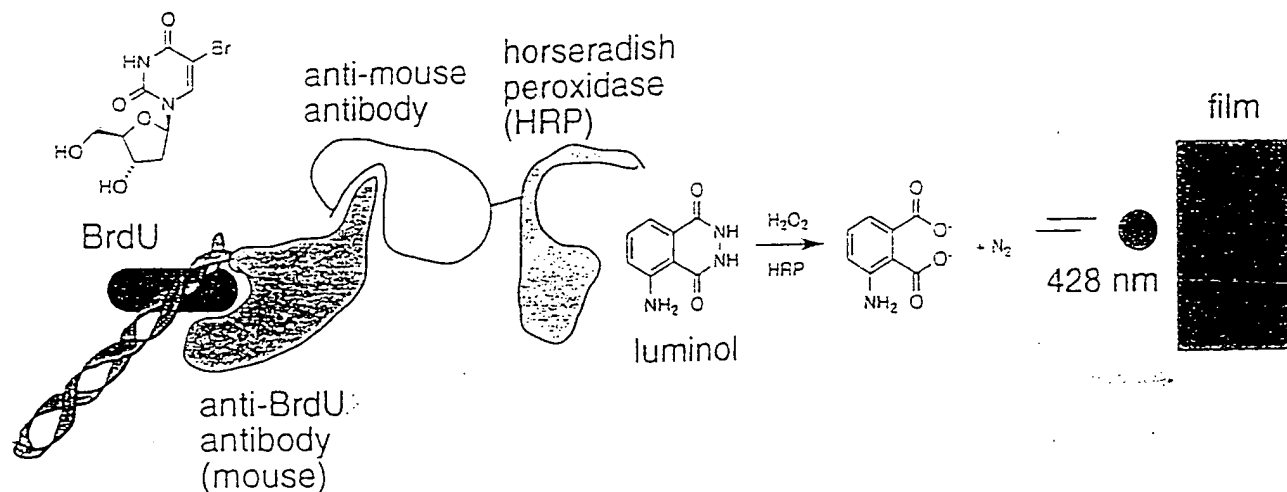
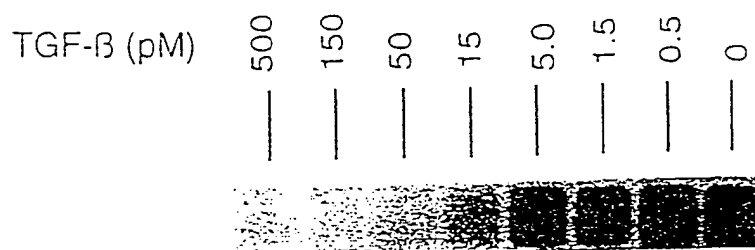


# An immunoassay for detecting DNA synthesis in high density arrays of mammalian cells

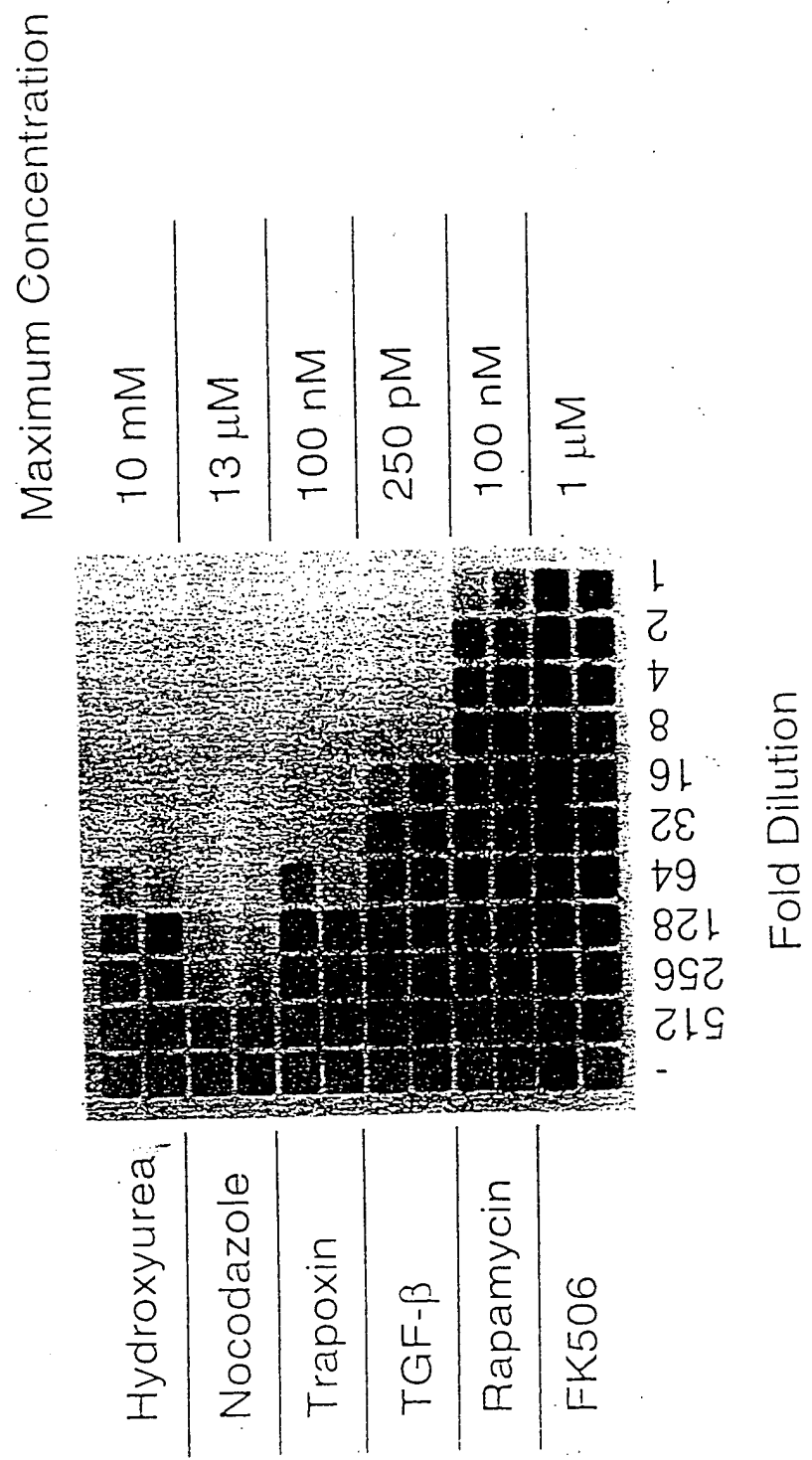
(a)



(b)



# Numerous Antiproliferative Agents Inhibit BrdU Incorporation

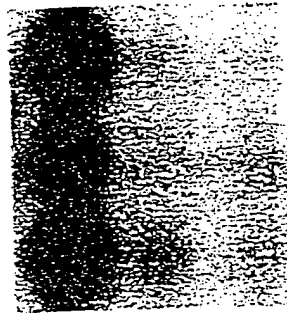


BrdU cyto blot on 2000 Mv1Lu cells, 43 hour treatment + 7 hours with BrdU

# *BrdU Incorporation Can be Efficiently Detected in 1536 Well Plates in 2 $\mu$ L Droplets*

Treatment  
BrdU TGF- $\beta$

+	-
+	+
-	-



Actual Size

36X Magnification

BrdU cytolot on 500 Mv1Lu cells, 28 hour treatment + 7 hours with BrdU

*BrdU incorporation is detected in 6144 well plates with 62 cells in 250 nL volumes*

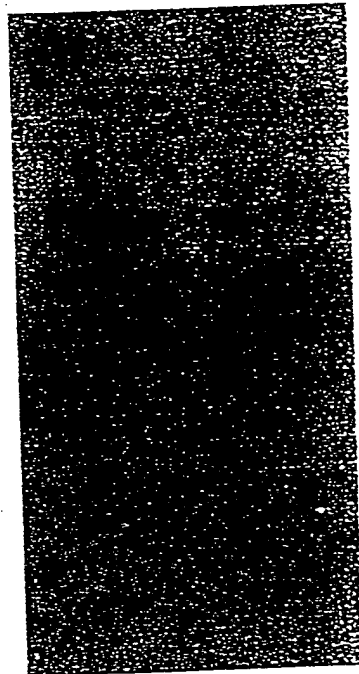
(a)

Original Size



(b)

25X Magnification



Number of cells per well

500 250 125 62 31

Treatment

BrdU TGF- $\beta$

+

-

+

+

-

-

BrdU cytotblot on Mv1Lu cells  
24 hour treatment with BrdU

(c)



No treatment



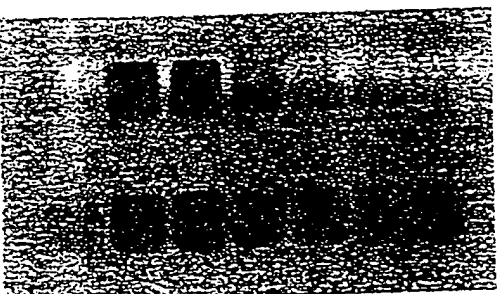
500 pM TGF- $\beta$

100X magnification

BrdU cytotblot on Mv1Lu cells  
26 hour treatment + 18 hours with BrdU

4.4

# *Hyperacetylation of histone H4 is detected with a cyto blot*



No treatment, no primary Ab

No treatment

0.5% serum

80 pM TGF- $\beta$

300 nM trichostatin A

100 nM trapoxin

250 nM nocodazole

anti-acetylated H4 blot

4X magnification

4000 A549 cells

00361576-072799

Fig 5

# *Phosphonucleolin is detected with the antibody TG-3 in a cyto blot*

TG-3 blot



No treatment



500 nM nocodazole  
24 hour treatment

500 1000 2000 4000

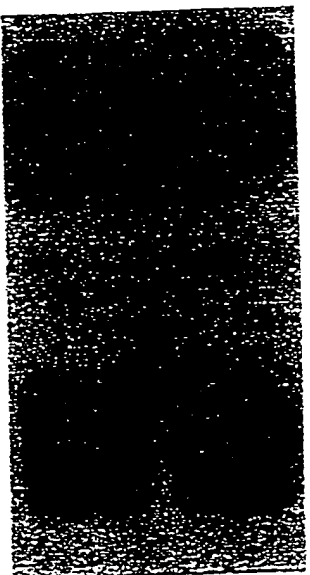
Number of cells (A549)

4X magnification

Fig 1a

# *Detecting phosphorylation of histone H3 as a marker of mitosis*

100 nM  
nocodazole      No treatment



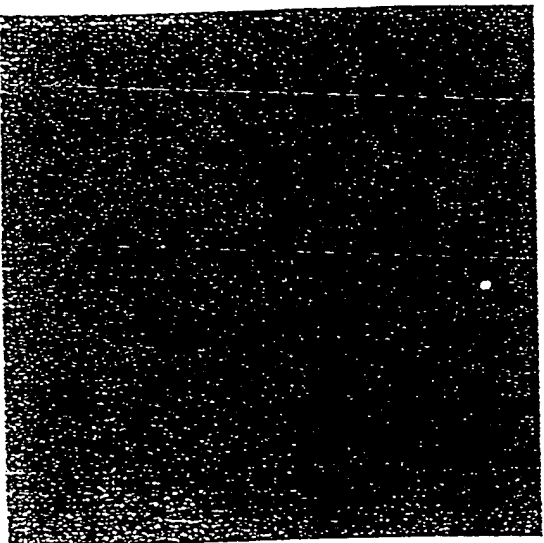
16 hour treatment, anti-phospho histone H3 mitosis marker

4000 A549 cells

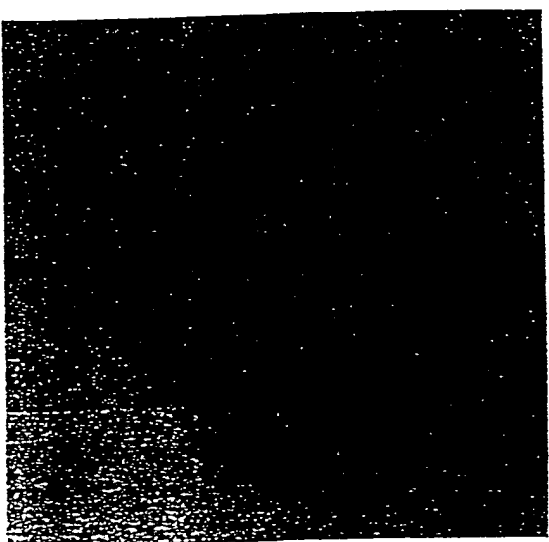
09361575-072799

6h  
Fig

# *Detection of phosphonucleolin with TG-3 in 1536 well plates with a cyto blot*



No treatment



250 nM nocodazole



size of one well

4X magnification  
TG-3 blot  
300,000 HeLa cells / mL

09351575-072799

Fig 28  
bc



00220-957960

anti-acetylated H4 antibody

HaCaT

A549



NT no 1° Ab

NT

0.5% serum

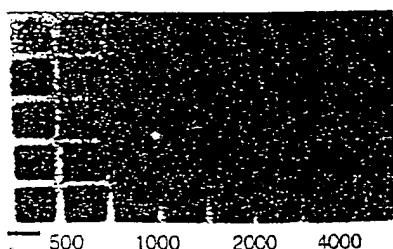
80 pM TGF- $\beta$

300 nM TSA

100 nM trap

250 nM ncdz

anti-acetylated H4 antibody



NT

4 hr

8 hr

12 hr

24 hr

trapoxin treatment

500 1000 2000 4000

Number of cells

anti-phospho histone H3  
antibody (mitotic marker)



NT

4 hr

8 hr

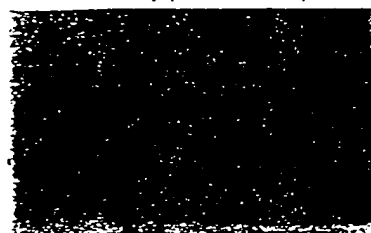
12 hr

24 hr

ncdz treatment

500 1000 2000 4000  
Number of cells

anti-phosphonucleolin  
antibody (mitotic marker)



NT

4 hr

8 hr

12 hr

24 hr

ncdz treatment

500 1000 2000 4000  
Number of cells

anti-phosphonucleolin  
antibody (mitotic marker)



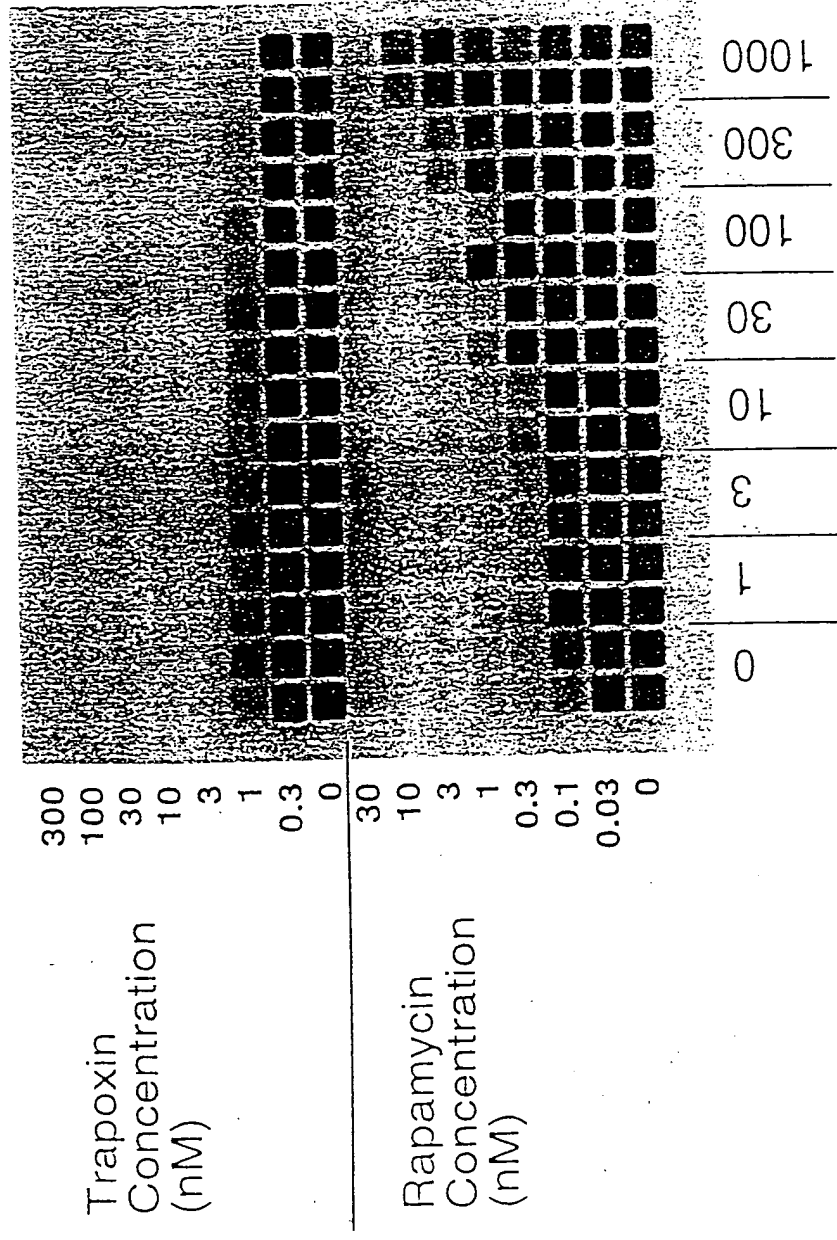
250 nM ncdz

NT

6144 well plate

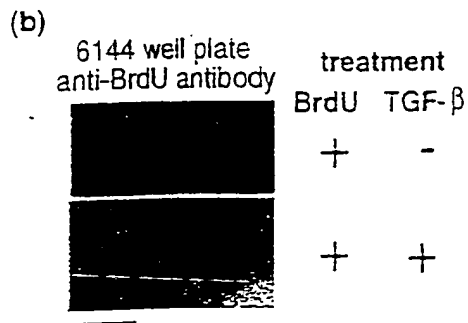
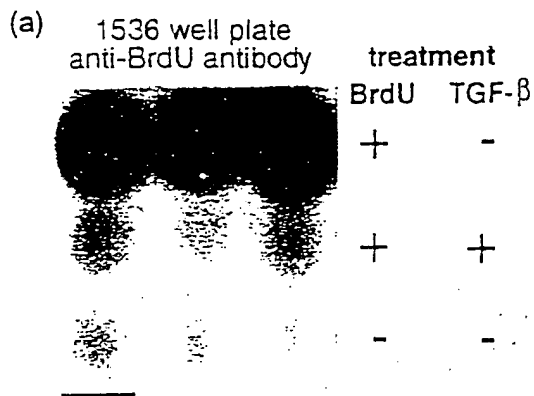
Fig 6d

# *FK506 Suppresses the Antiproliferative Effect of Rapamycin but not Trapoxin*

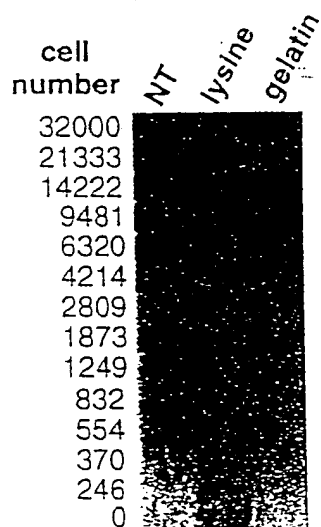


FK506 Concentration (nM)

BrdU cyto blot on 2000 6F cells, 44 hour treatment + 7 hours BrdU treatment

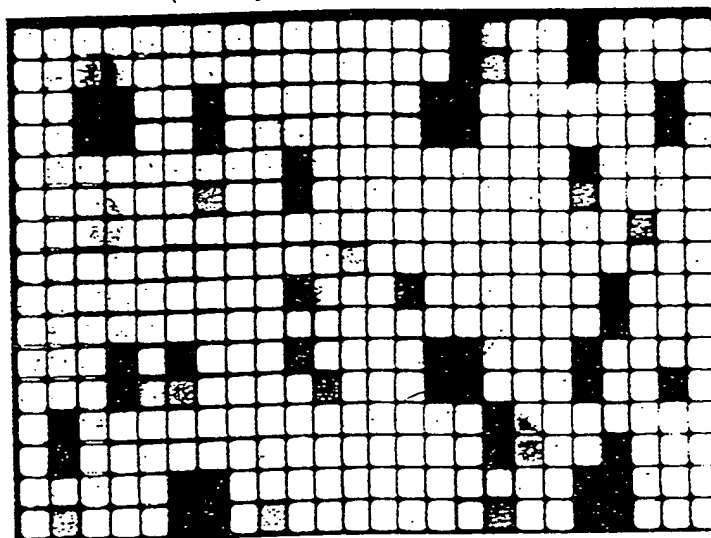


(c) cytotblot on mouse embryonic stem cells

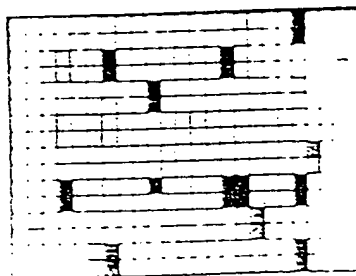


384 well plate  
anti-BrdU antibody

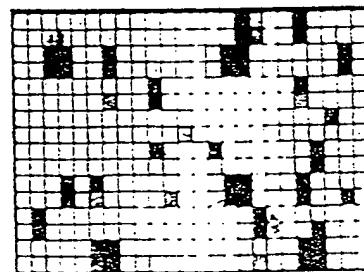
(d) anti-BrdU cytotblot after treatment with 192 marine sponge extracts (overlay of p53<sup>-/-</sup> plate and p21<sup>-/-</sup> plate)



red = selective BrdU incorporation inhibitor for p21<sup>-/-</sup>, p53<sup>+/+</sup> genotype  
green = selective BrdU incorporation inhibitor for p21<sup>+/+</sup>, p53<sup>-/-</sup> genotype  
black = nonspecific BrdU incorporation inhibitor  
white = no effect on DNA synthesis



p53<sup>-/-</sup> MEFs



p21<sup>-/-</sup> MEFs

Fig 8

## Effect on mink lung cells

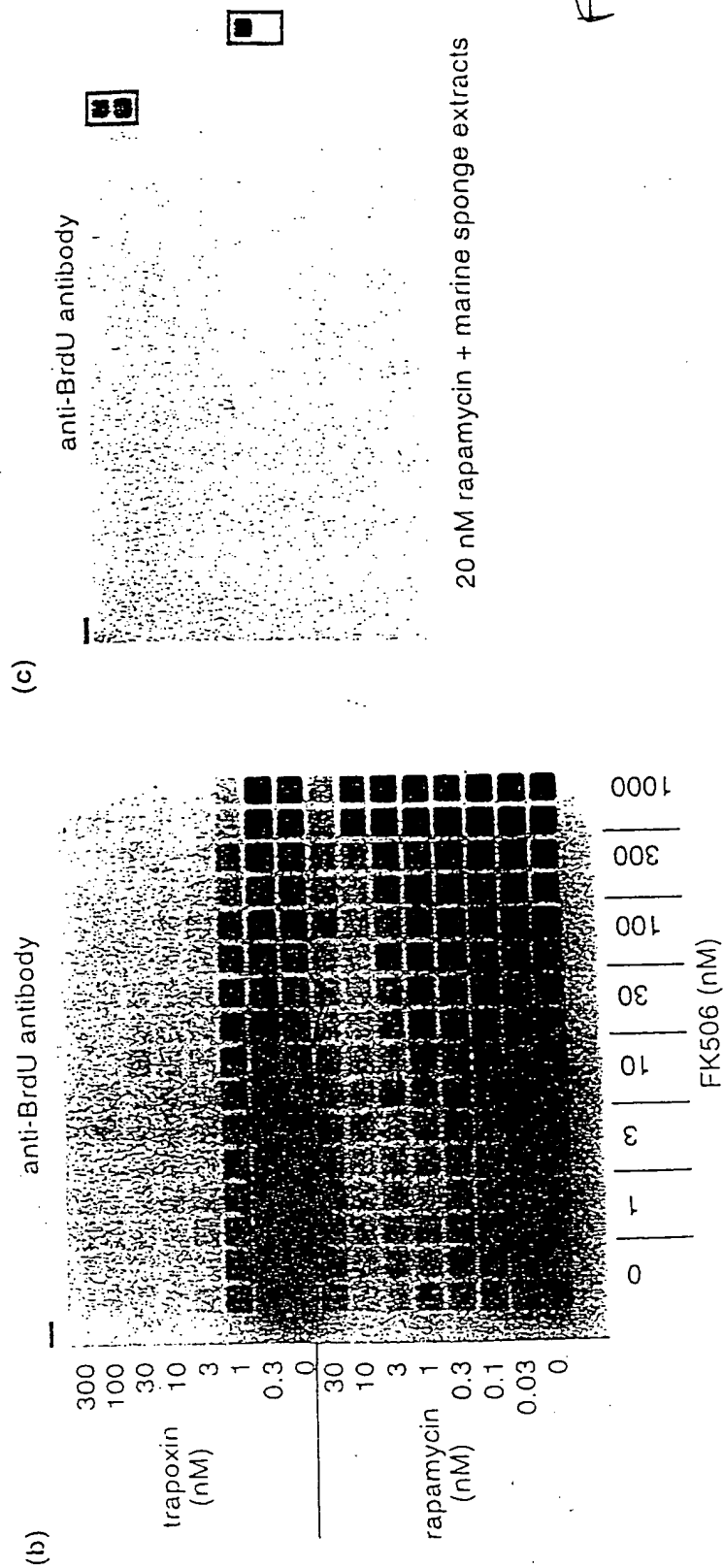
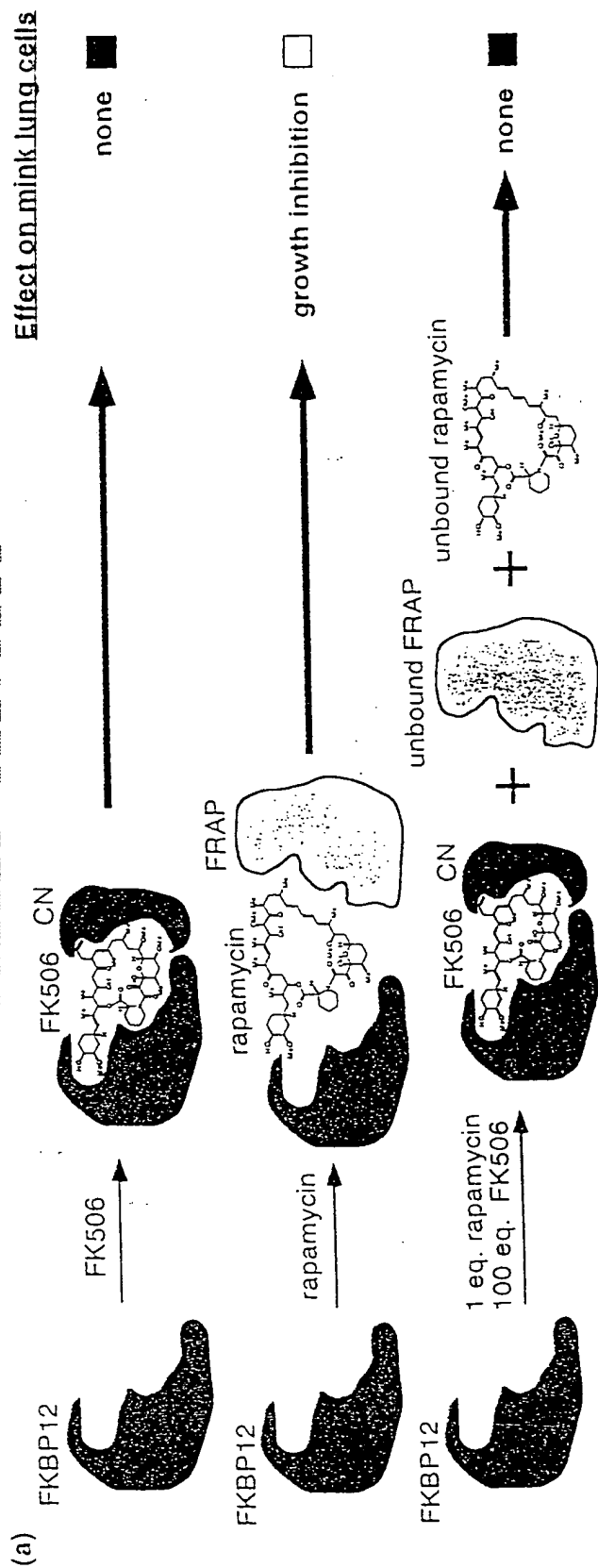


Fig. 9.

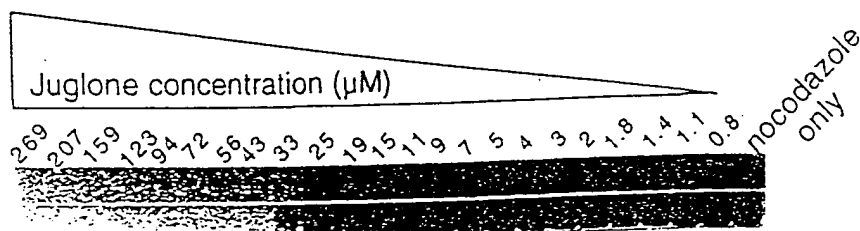
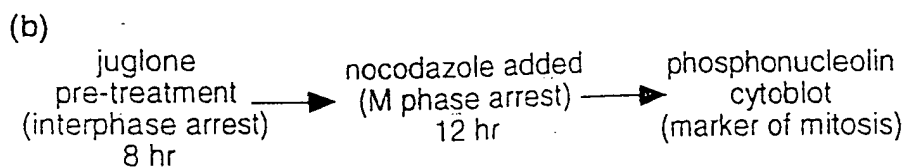
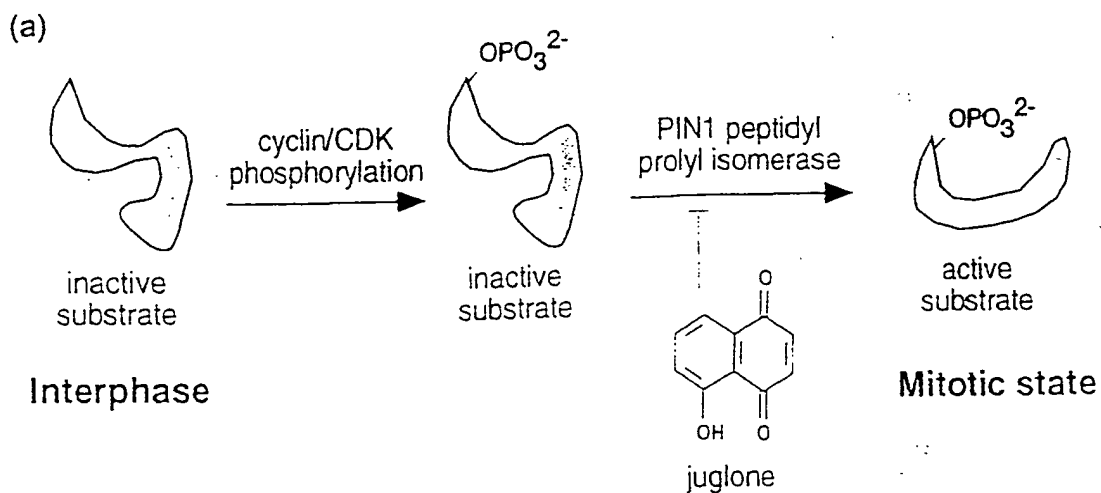
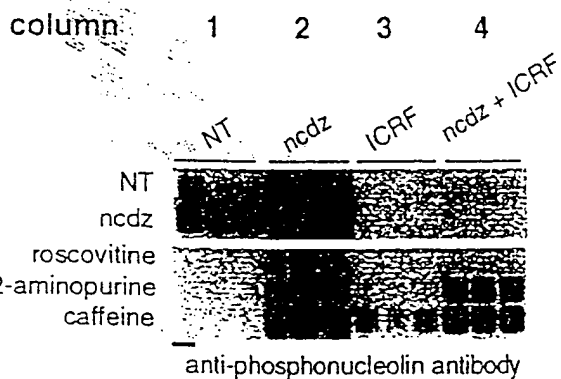
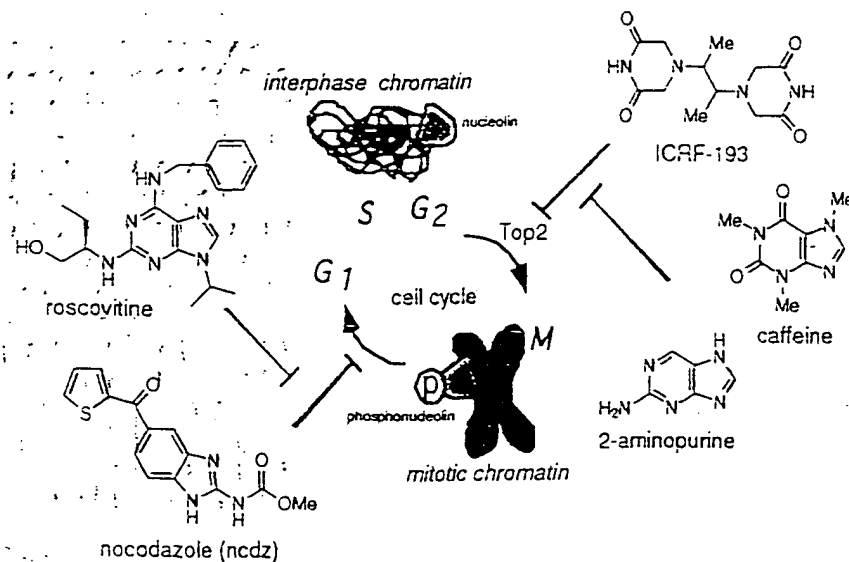


Fig 10

(a)



(c)

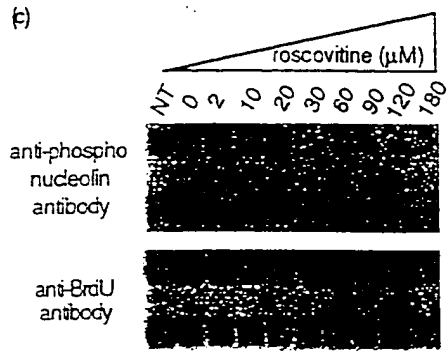
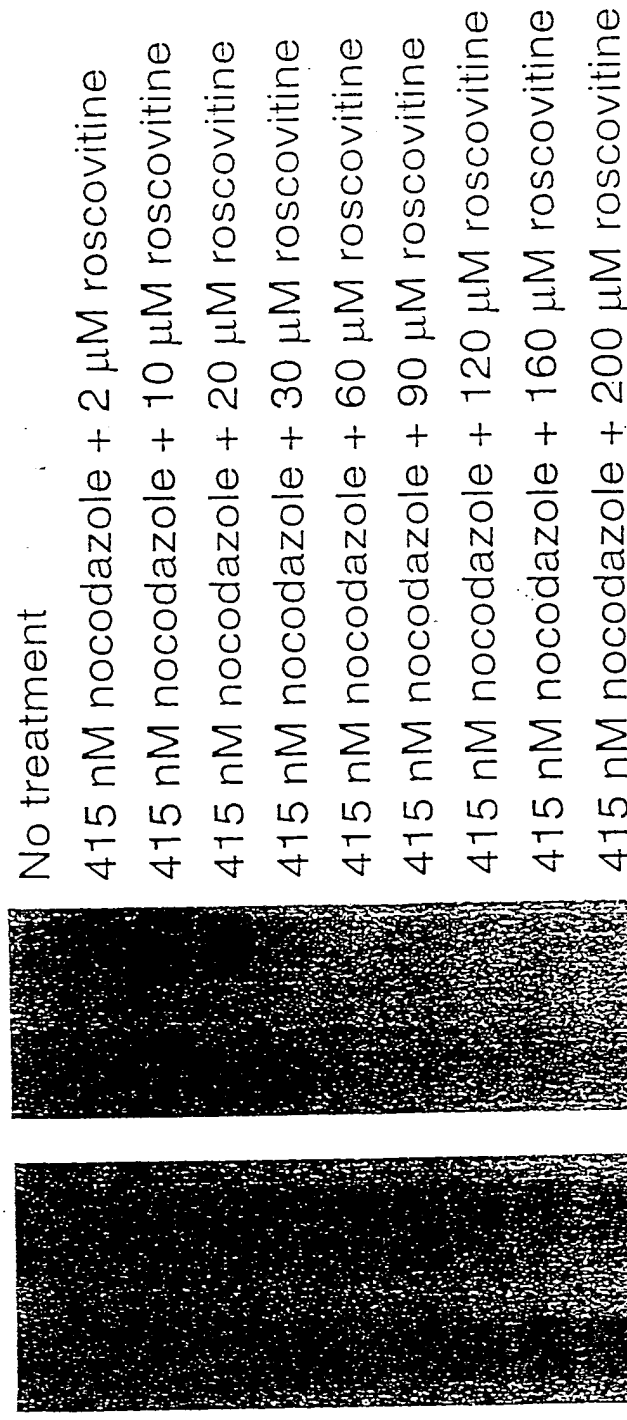


Fig 11

# *Nocodazole prevents BrdU incorporation and induces the accumulation of phosphonucleolin*

*Roscovitine suppresses only the latter effect*



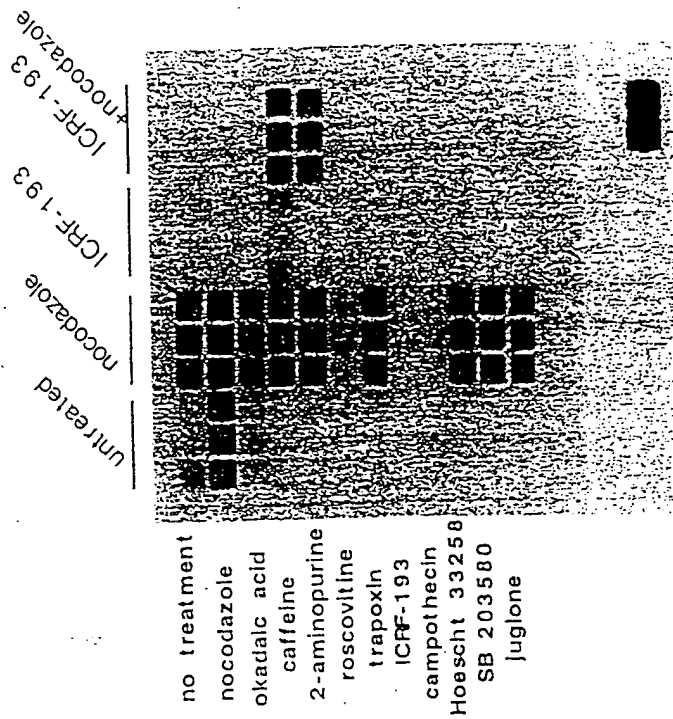
anti-BrdU      TG-3

4000 HeLa cells  
4X magnification

11d

Fig 7

# *Suppression of a DNA damage-independent topoisomerase inhibitor-induced G2-checkpoint arrest by caffeine and 2-aminopurine*



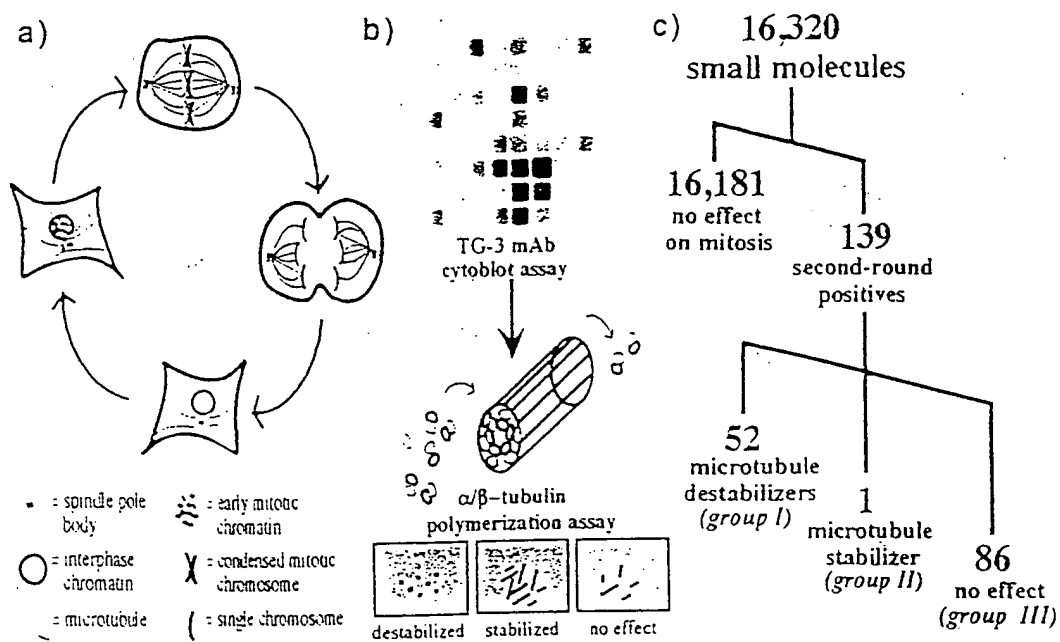
TG-3 blot

18 hr treatment

A549 cells 384 well plate  
~6000 cells/well

Fig 11e



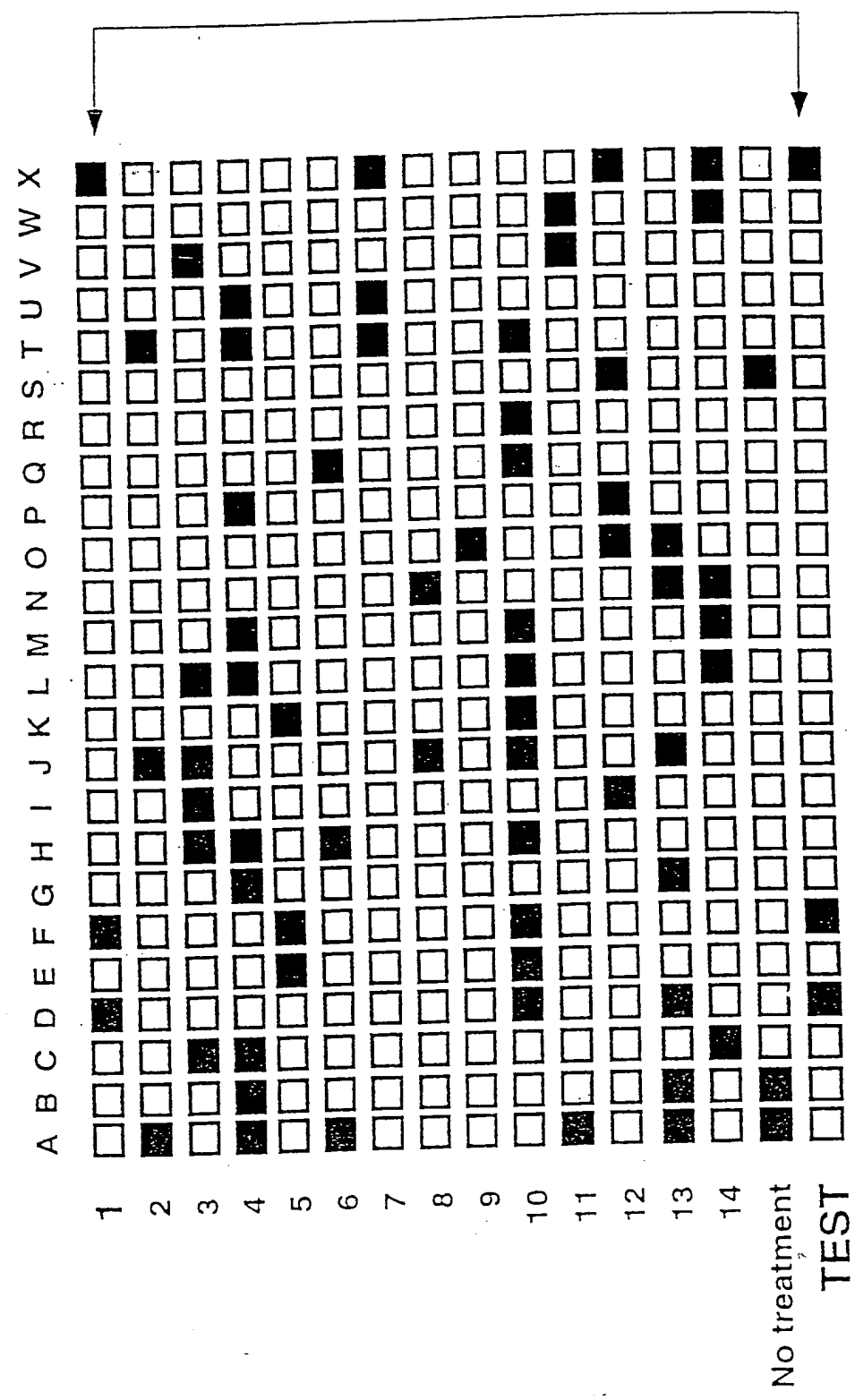


**Figure 1.** Screening for small molecules that affect the mammalian cell division cycle. a) Schematic of cell cycle events involved in mitotic chromosome segregation. b) summary of screening steps. c) division of small molecules into three groups based on their effects on the stability of purified microtubules.

Fig. 12

66220-929200

# Functional Fingerprinting of a Test Compound with 24 Different Antibodies



13  
Fj

002009034500

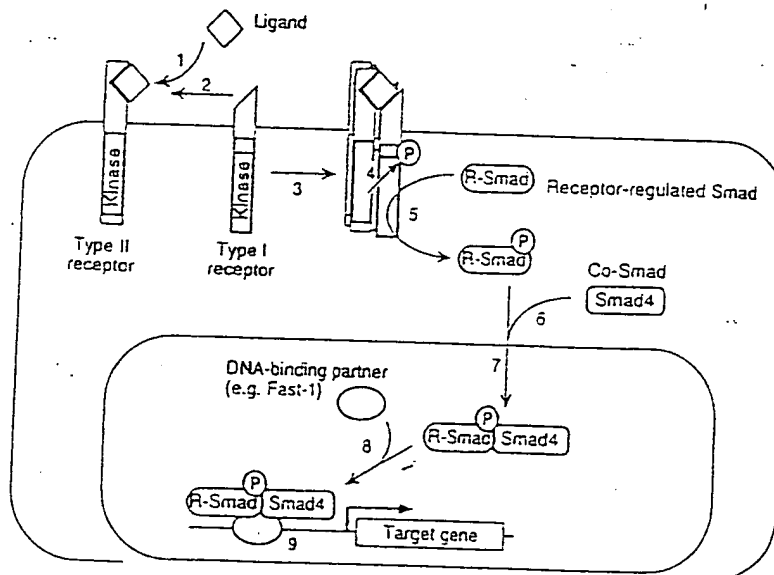


FIGURE 14

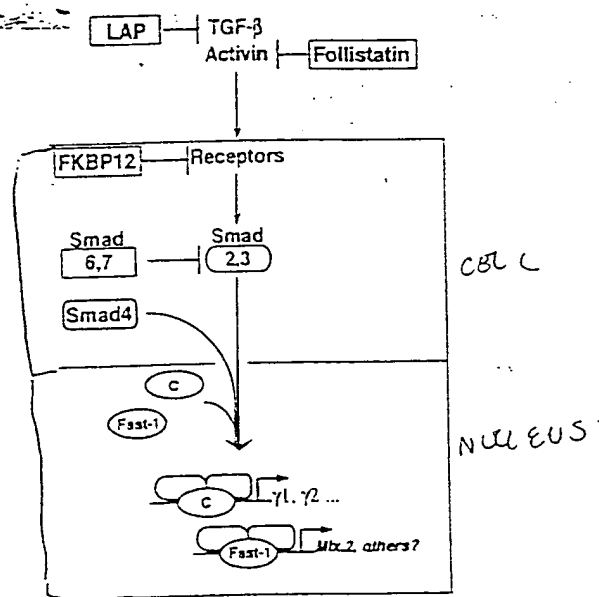


FIGURE ~~14~~  
15

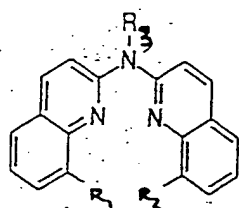


FIGURE ~~2~~  
16

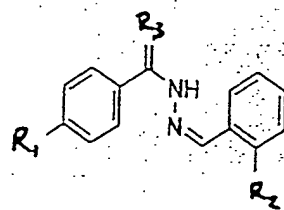
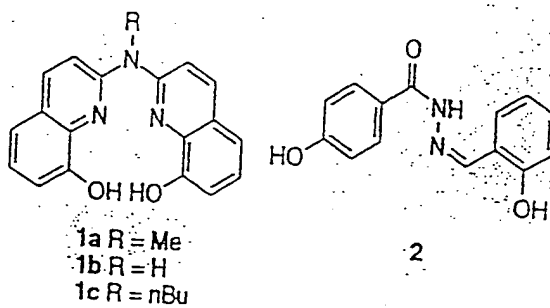


FIGURE ~~7A~~  
17

FIGURE 18



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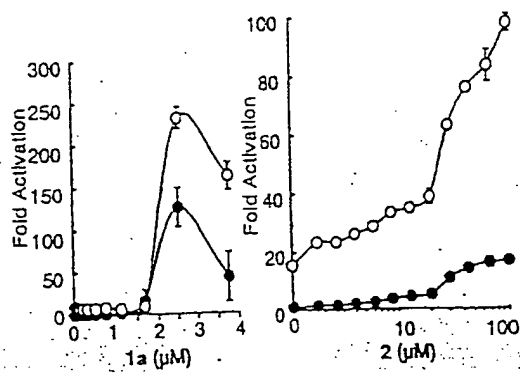
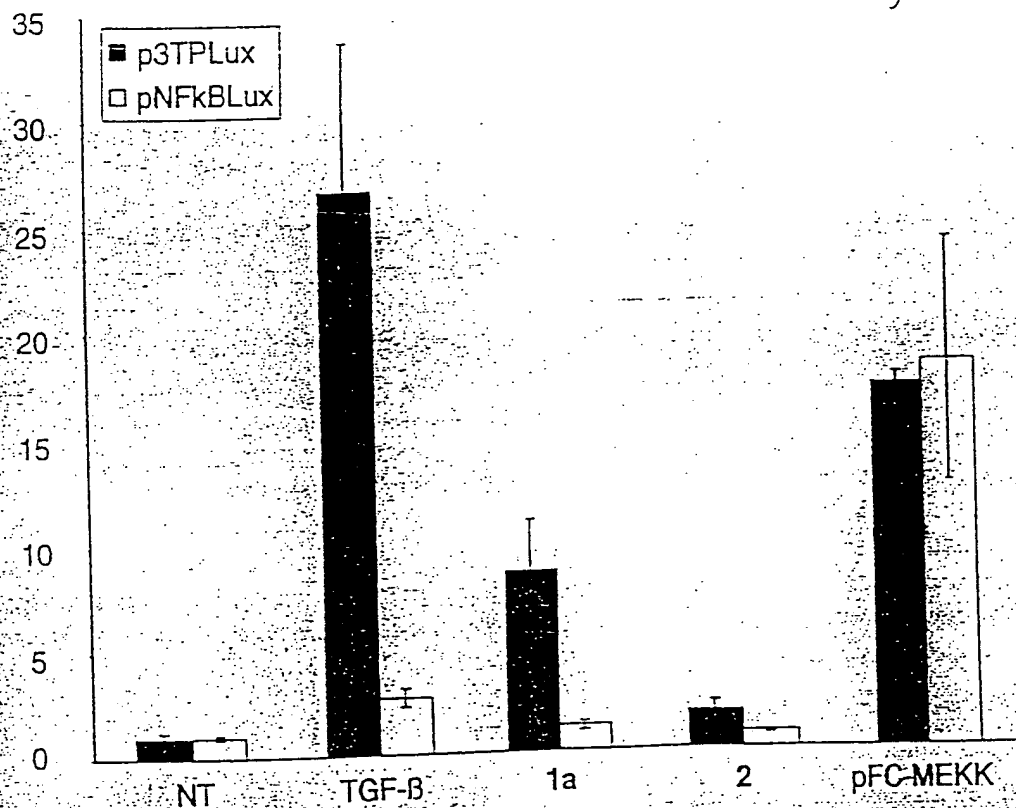


Figure ~~18~~  
19



Fig. 20



**Figure 22.** Reporter gene specificity of 1a and 2.  $1 \times 10^5$  Mv1Lu mink lung epithelial cells were transiently transfected with 400 ng reporter gene (p3TPLux or pNFkBLux) alone or with 50 ng pFC-MEKK using DEAE dextran [ref] in 12-well dishes and subsequently cultured in mink medium with 10% FBS for 23 hours. The cells were then treated with nothing (NT), 400 pM TGF- $\beta$ 1 (TGF-B), 2.5  $\mu$ M 1a, or 50  $\mu$ M 2 in mink medium with 0.2% FBS. After 24 hours, luciferase activity was measured as described previously [ref].

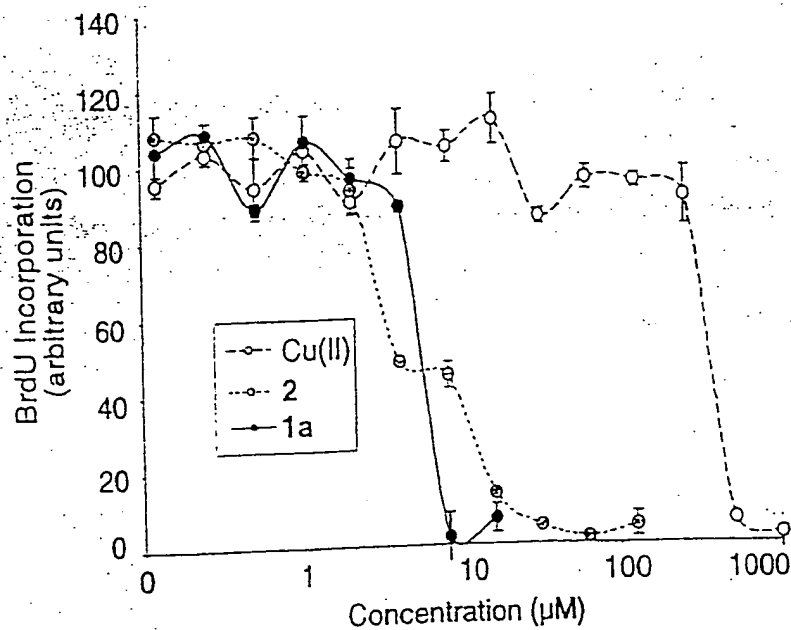


Figure 81: 1a, 2 and Cu(II) inhibit BrdU incorporation in mink lung epithelial cells. 2000 cells were seeded in each well of a 384 well plate in the presence of the indicated concentrations of reagents. The BrdU cyto blot assay was performed as described previously [ref].

Fig. 21

Fig. 22

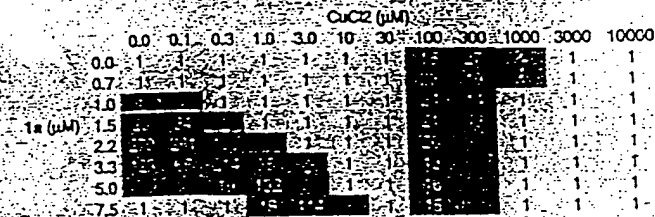
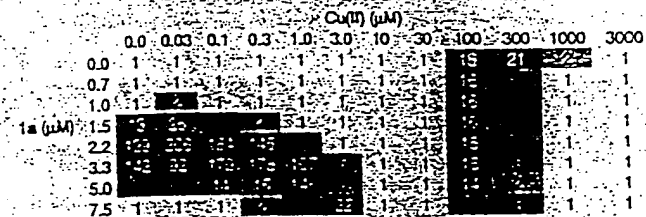
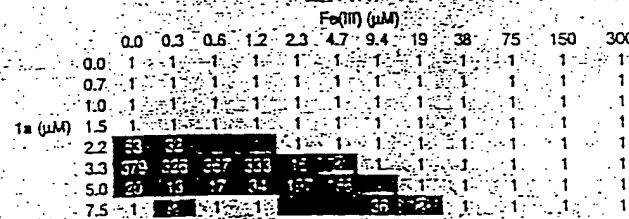
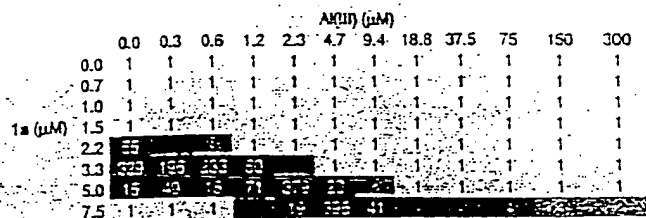
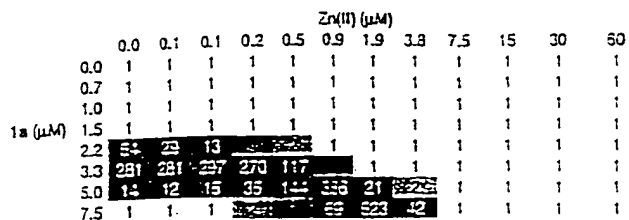
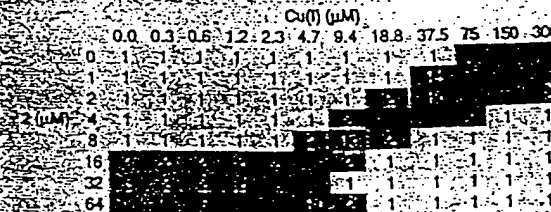
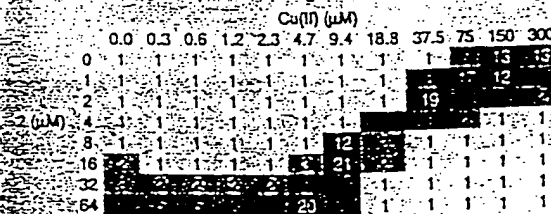
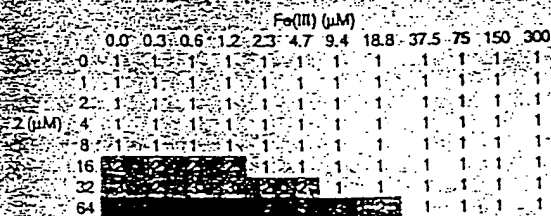
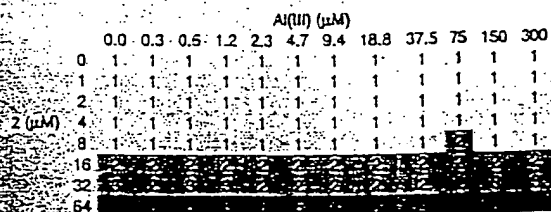
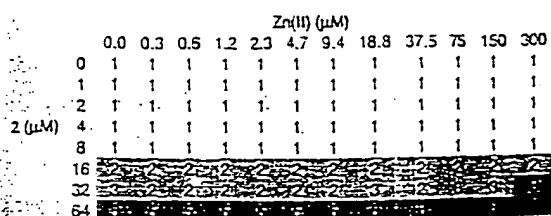


Fig. 22. Effect of metal ions on the activity of 1a and 2. Two-dimensional dose-responses are shown of metal against compound. The interior boxes indicate fold activation and are colored according to the following greyscale: 0-1.5 = white, 1.5-3 = light grey, 3-5 = grey, 5-12 = dark grey, >12 = black. All experiments were performed in quadruplicate.

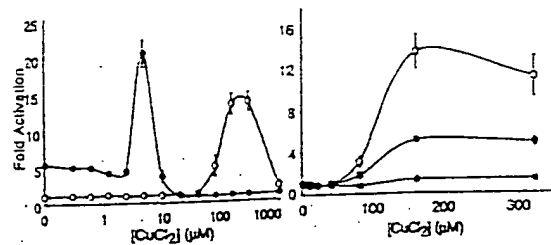


Figure ~~22~~  
23

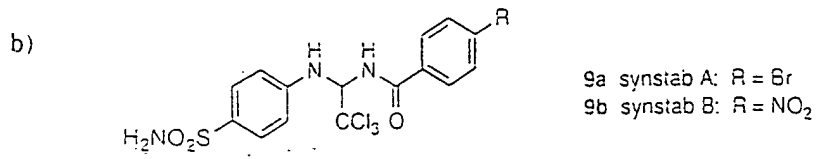
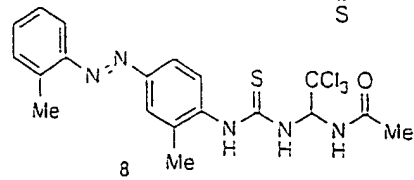
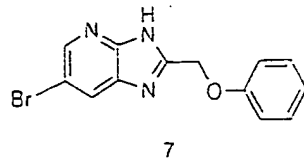
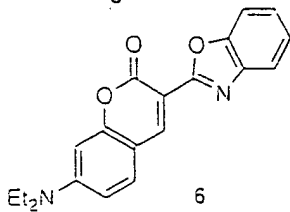
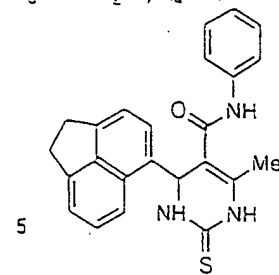
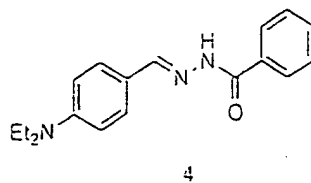
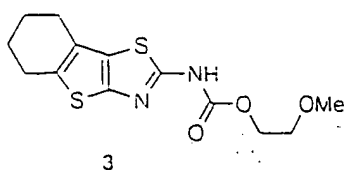
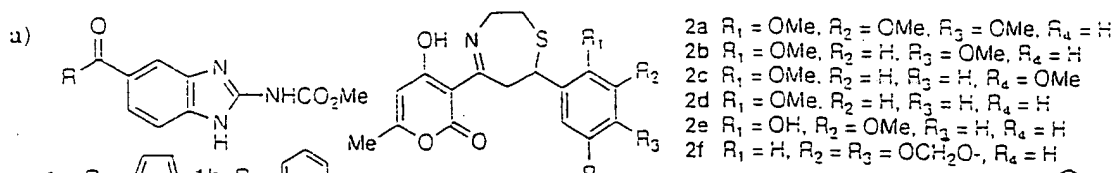


Fig. ~~23~~ 24

Cc1cc(O)c2c(c1)oc(=O)c2C3=NC4CCSC4C3c5cc(R1)cc(R2)cc5R3

- 2g  $R_1 = H, R_2 = OMe, R_3 = OMe, R_4 = OMe$   
 2h  $R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = OMe$   
 2i  $R_1 = OMe, R_2 = OMe, R_3 = H, R_4 = H$   
 2j  $R_1 = H, R_2 = H, R_3 = OMe, R_4 = H$   
 2k  $R_1 = H, R_2 = OMe, R_3 = OH, R_4 = H$   
 2l  $R_1 = H, R_2 = OH, R_3 = H, R_4 = H$   
 2m  $R_1 = OH, R_2 = Cl, R_3 = H, R_4 = Cl$   
 2n  $R_1 = H, R_2 = H, R_3 = Cl, R_4 = H$   
 2o  $R_1 = H, R_2 = Br, R_3 = H, R_4 = H$   
 2p  $R_1 = H, R_2 = H, R_3 = isopropyl, R_4 = H$   
 2q  $R_1 = H, R_2 = H, R_3 = Me, R_4 = H$

Fig. ~~24~~ 25

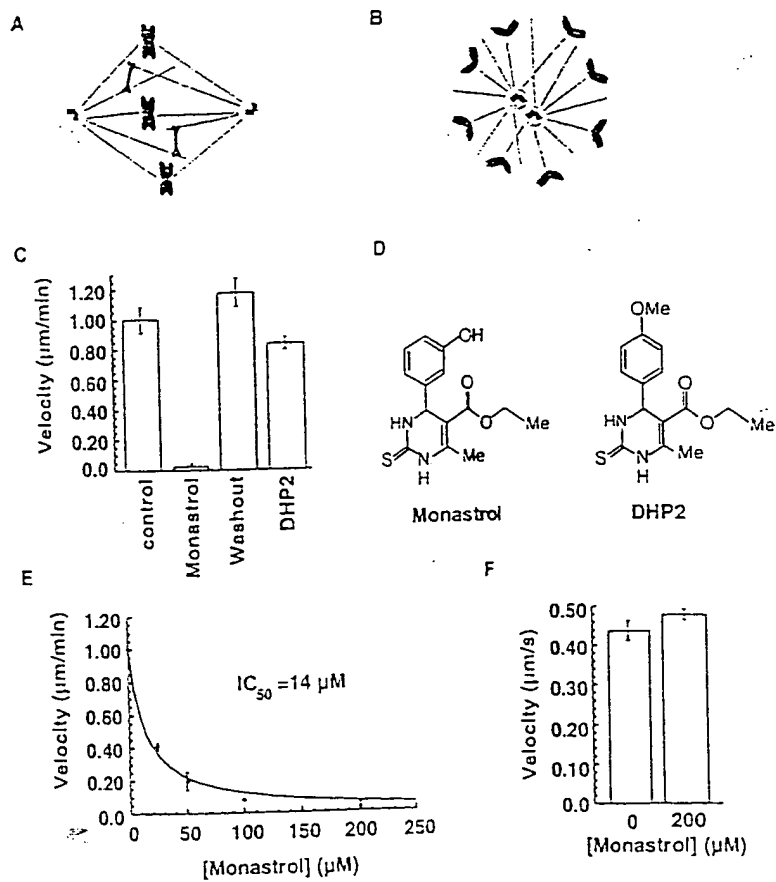


Fig. 25 26

NS(=O)(=O)c1ccc(NC(C(Cl)(Cl)Cl)NC(=O)c2ccc(Br)cc2)cc1CN1C=C(C(=O)Nc2ccccc2)c3ccccc13CC(C)c1ccc(NC(=O)/C=C/c2ccccc2)cc1CCOC(=O)C1=C(C)NC(=S)NC1C2=CC=CC=C2OCOc1ccc(cc1)C2(Cc3ccccc3)OC(C2)C4OC(=O)N5C(=O)C(=O)N5C4OS(=O)(=O)c6ccccc6

3

Figure ~~25~~ 27